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Ju-Wen Chen  
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## INTRODUCTION

Combination chemotherapy is one of the important strategies in cancer treatments. This is based on the observation that administering certain drugs together is more effective than giving individual drugs separately. Although the reason for such an effect is not understood, it may be related to the synergistic effect of their binding to macromolecules. Consequently, studies on the interplay among drugs capable of binding to different regions of DNA will be of considerable interest. Understanding the synergism of drugs at the molecular level may have important implication for designing more effective chemotherapeutic strategies in breast cancer treatments. Our proposal focuses on the sequence specific binding and synergistic effects of three drugs having distinctly different binding modes: actinomycin D (ACTD), an intercalator with GpC sequence preference; chromomycin A<sub>3</sub> (CHR), a guanine specific minor groove binder; and distamycin A (DST), an A•T specific minor groove binder. In order to investigate the possible synergistic effects of drugs on DNA binding, it is essential that binding characteristics of each individual drug such as binding affinities, sequence specificities, and kinetic behaviors be thoroughly elucidated. Consistent with the Statement of Work outlined in the proposal, these were done during the previous years. Attempts have now been made to commence the synergistic studies during the past year.

Sequence-specific recognition of DNA molecules by proteins and small molecules is an important component in the regulation of many biological processes. Understanding the structure, sequence specificity, and forces responsible for the binding of antibiotics to DNA molecules is an important first step in the design of new drugs and sequence-specific probes. However, elucidation of sequence specific binding of each individual drug is not only of importance in its own right but these results will also be crucial in serving as bases for designing oligonucleotides for our subsequent synergistic studies. Thus, a brief review on relevant results of these drugs is in order.

ACTD is a chromopeptide antibiotic which consists of a 2-aminophenozazin-3-one chromophore and two identical pentapeptide side chains. It has been well established that this drug prefers duplex DNA and binds via intercalation of the planar chromophore, preferably at the GpC sequence, with the two pentapeptide rings resting on the minor groove. X-ray studies (Sobell et al., 1971; Kamitori & Takusagawa, 1992) have clearly shown that its duplex and GpC sequence preference is the consequence of the ability of the carbonyl oxygens and the NH groups of the L-threonine residues to form hydrogen bonds with the 2-amino group and the N(3) of guanines on both sides of the intercalated drug. These essential drug-DNA hydrogen bonds are protected by the cyclic pentapeptides, which effectively shield them from solvent exposure. The size of the four-base-paired binding site suggests that the binding characteristics of ACTD to the GC site may be affected by the adjacent flanking base pairs. Consequently, ACTD binding to oligomers containing XGCY = TGCA, AGCT, CGCG, and GGCC was investigated by us via equilibrium, kinetic, and thermal denaturation studies (Chen, 1988). The results indicate that despite the presence of a GC dinucleotide sequence, -GGCC- exhibits a much weaker binding affinity towards ACTD than the other three tetranucleotide sequences. Binding constants estimated from Scatchard plots indicate that binding to the -GGCC- site is at least an order of magnitude weaker than binding to -CGCG- and -AGCT-, which in turn is somewhat weaker than binding to the -TGCA- sequence. At 18.5 °C, ACTD dissociates

from a decamer containing -TGCA- roughly 4 times slower than dissociation from those containing -CGCG- and -AGCT-sequences and more than two orders of magnitude slower than that from -GGCC-. These results were also supported by the melting results in which a stronger binding and slower dissociating site exhibits a larger increase in melting temperature upon ACTD binding.

CHR is an antibiotic of the aureolic class which contains an aglycon chromophore with di- and trisaccharide (2,6-dideoxyhexapyranose) side chains attached on opposite sides of the chromophore. The antitumor activity of these drugs is believed to be the consequence of the ability to bind to duplex DNA to result in an inhibition of DNA-directed RNA synthesis. Recent NMR studies on the CHR-d(TTGGCCAA) (Gao & Patel, 1989; Gao et al., 1992) and CHR-d(AAGGCCTT) (Gao & Patel, 1990) complexes in Mg<sup>++</sup>-containing solutions have established unequivocally that CHR binds as a Mg<sup>++</sup>-coordinated dimer at the minor groove of the central GGCC segment of the duplex and have revealed the structural basis for the sequence specificity at the 5'GpC3' step. The detailed structural information available on the binding to -GGCC- and -CGCG sequences (Sastry & Patel, 1993) has provided considerable insights into the structure and dynamics of aureolic acid-DNA interactions. Hence, a systematic study on the binding specificity to other sequences via synthetic oligonucleotides is of value. To this end, systematic kinetic, equilibrium binding, melting, and electrophoretic studies were carried out with oligonucleotides to determine the sequence specificity of chromomycin A<sub>3</sub> (CHR) binding to DNA at the self-complementary tetranucleotide level (Liu & Chen, 1994). Results indicate that the binding preferences for CHR are in the order -GGCC- > -CGCG- > -GCGC-, -CCGG- > -AGCT- > -ACGT-, -TGCA- > -TCGA-. Detergent-induced drug dissociation studies revealed that CHR dissociates very slowly from both -GGCC- and -CGCG- sequences, with the former being measurably slower than the latter which in turn is at least an order of magnitude slower than the rest of the sequences. Thermal denaturation measurements indicate that the binding of CHR stabilizes the DNA duplex, with -GGCC- and -CGCG- exhibiting the largest effects. Results of gel electrophoretic retardation experiments support our general findings on the relative binding order. Our experimental results support earlier NMR findings by other researchers implicating the preference of aureolic acid drugs at the 5'GpC3' step and further reveal significant modulations by the adjacent base pairs.

DST has received considerable attention as a model of sequence specific nonintercalative DNA binding molecules. It is a potent antibacterial, antiviral, and antineoplastic agent whose pharmacological activity has been correlated to its ability to bind to DNA. It forms non-covalent complexes with duplex DNA in the minor groove and exhibits considerable preference for the AT-rich domains such as promoter regions. This antibiotic, thus, acts as a template poison and inhibits DNA-dependent polymerase activities. Its binding affinities are in some cases significantly larger than those of typical intercalating drugs. Recent years have further seen the upsurge of interest in using DST as a model for the DNA minor groove binding with A•T preference. Several NMR studies of 1:1 DST-DNA complexes have provided insights into both the specificity and the forces responsible for the tight binding of this drug. The structure of the DST-d(CGCGAATTCGCG)<sub>2</sub> complex was determined by a combination of 2D NMR experiments and molecular mechanics calculations (Pelton & Wemmer, 1988). It was found that the minimal binding site consists of just four A•T base pairs and that DST fits snugly into the 5'-AATT-3' minor-groove binding site. Most interestingly, NMR studies have further indicated that binding sites of at

least five base pairs in length can accommodate two DST molecules side-by-side in an anti-parallel orientation (Pelton & Wemmer, 1989; 1990). In this 2:1 complex, each ligand preserves all the molecular recognition elements of minor groove binders. The extent of binding cooperativity (i.e., the ease of forming a complex of 2:1 vs 1:1 DST-duplex) depends strongly on the DNA sequence. We have carried out a more systematic study on the sequence-specific binding of DST (Chen & Sha, 1998). DNA binding modes of DST were investigated via comparative binding studies with oligomeric duplexes of the form d(GCG-X-GCG)•d(CGC-Y-CGC), where Y is complementary to X and X = 4- or 5-base binding site. It was found that 1:1 and 2:1 drug:duplex complexes exhibit distinctly different circular dichroic (CD) spectral characteristics and can, thus, serve as diagnostic tools for binding mode differentiation. CD intensity profile at 265 or 275 nm as a function of drug to DNA ratios can reveal the extent of binding cooperativity for the 2:1 complex formation (i.e., the relative binding affinities of 2:1 vs. 1:1) at a 5-base-paired binding site. Comparative studies (Chen & Sha, 1998) lead to the following ranking for the binding cooperativity of these sites: AAGTT, ATATA ≥ AAACT > AATAA, AAATA, AAAGT > AATAT > TAAAA ≥ AAATT ≥ AAAAA ≥ ATAAA, AAAAT. The plausibility of this ordering is strengthened by its agreement with the ranking established by earlier NMR studies on some of the sequences. The significantly slower DST dissociation kinetics of the 2:1 complexes as compared to those of 1:1 made the kinetic measurements of SDS-induced dissociation by the stopped-flow technique possible. The results indicate that the AAGTT site exhibits the slowest DST dissociation rate, with a characteristic time of 35 seconds. The rates of dissociation in general correlate reasonably well with the cooperativity order found via equilibrium CD measurements (the higher the binding cooperativity the slower the rate of dissociation). Base sequence specific binding of DST was also found for the 1:1 complex formation at the 4-base-paired sites, with AAAAA, TTTT, ATTT, and AAAT sequences exhibiting the highest binding affinities.

Based on these sequence-specific binding studies of each individual drug, oligonucleotides of appropriate lengths and sequences can now be designed to carry out the synergistic binding studies.

## BODY

### Experimental methods, assumptions, and procedures.

Oligomers of the d(ATATA<sub>X</sub>CYTATAT) motif were employed to carry out our initial synergistic studies, where X = A, C, G, or T and Y is complementary to X. The rationale for the choice of these oligomers stems from the fact that both ACTD and CHR prefer a GpC sequence whereas DST prefers A•T base pairs. Thus, it is expected that ACTD or CHR will bind at the -XGCY- central region while DST will bind at the two AT-rich regions at both ends. ATATA sequence was chosen since the alternating AT sequence of 5 base pairs have been shown to bind DST tightly with a 2:1 drug to duplex binding stoichiometry. Studies were also made with oligomers of the form d(CGCGATATAAGGCC)• d(GGCCTATATCGCG) and d(GGCCATATAACGCG)• d(CGCGTATATGGCC). These oligomers will lead to ACTD binding on both ends of the duplex whereas the DST will bind at the center. It is anticipated that with ATATA at the center, DST binding will predominantly be of 2:1 drug to duplex binding mode. The

rationale for studying these two distinct systems stems in part from the fact that due to the fraying motion, DST binding at duplex ends may be weaker and may exhibit binding mode somewhat different from binding at the duplex center where a 2:1 binding mode has been established.

*Equilibrium Binding Titrations via Absorbance Spectral Changes.* ACTD exhibits an absorption maximum near 440 nm in buffer solutions and without the presence of DNA. Progressive additions of DNA oligomeric stock result in considerable hypochromic effects of this band and slight bathochromic shifts to result in some hyperchromic effects around 480 nm. Absorbance differences between 427 and 480 nm were, thus, used to construct the binding isotherms and Scatchard plots. From these plots, the binding parameters were extracted. Similar titrations were then made in which both the starting ACTD drug solution and the DNA oligomer stock contain 8  $\mu$ M DST so that the DST concentration during titrations remains unchanged.

DST exhibits an absorbance maximum at 303 nm when free in solutions. Successive additions of DNA lead to slight bathochromic shift and intensity enhancements near 330 nm. Thus, absorbance changes at 350 nm (to avoid interference from the residual DNA absorbance) were used to construct binding isotherms and to obtain binding parameters. Similar DST titrations in the presence of constant 5  $\mu$ M ACTD concentration were then carried out to reveal the effect of ACTD on the DST binding. Ligand binding to DNA usually results in a melting temperature increase so that the extent of the increase can give a qualitative measure of the binding affinity. Melting measurements were made with DNA alone and in the presence of one or two drugs.

Absorption spectra were measured with Cary 1E spectrophotometric system. Spectral titrations were carried out at 20 °C by starting with a drug solution followed by progressive additions of the oligomer stock. Thermal denaturation experiments of 40  $\mu$ M oligomer in the absence and in the presence of appropriate concentrations of ACTD and DST were carried out with 1-cm semimicro cells by monitoring the absorbance at 275 nm. A heating rate of 0.5 °C/min was maintained by the temperature controller accessory. Melting temperatures were then deduced via differential melting profiles. Absorbance kinetic measurements were made by using a stirrer accessory with 427- and 453-nm monitoring for the ACTD association and SDS-induced dissociation, respectively. Kinetic rate parameters were extracted using a nonlinear least-squares fit program.

*Equilibrium Binding Titrations and Spectral Characterization of DST Binding Modes via Circular Dichroism.* DST is not optically active when free in solutions. In the presence of DNA, however, a strong positive CD band with a maximum near 330 nm is induced. Thus, induced CD intensities at 330 nm can be used to construct binding isotherms via CD spectral titrations. CD spectra were measured at room temperature by a Jasco J-500A recording spectropolarimeter using water-jacketed cylindrical cells of 2-cm pathlength. CD titrations were carried out with 80  $\mu$ M DNA nucleotide followed by progressive additions of the drug stock solution. CD spectra were measured from 230 to 380 nm.

Since DST is not very stable in aqueous solutions, stock solutions were prepared immediately before use. Drug concentrations were determined using extinction coefficients of  $\epsilon_{303} = 34,000$  and  $\epsilon_{440} = 24,500 \text{ cm}^{-1}\text{M}^{-1}$  for DST and ACTD, respectively. Synthetic oligonucleotides were purchased from Research Genetics, Huntsville, AL, and used without further purification. Concentration of these oligomers (per nucleotide) were determined by measuring the absorbances at 260 nm after melting, with use of extinction

coefficients obtained via nearest-neighbor approximation using mono- and dinucleotide values tabulated in Fasman (1975). All experiments were carried out in 10 mM tris/borate buffer of pH 8 containing 0.1 M NaCl and 1 mM MgCl<sub>2</sub>.

## Results and Discussion

*DNA Binding of ACTD in the Absence of DST.* In order to investigate the effect of DST on the ACTD binding of DNA, the DNA binding propensity of ACTD in the absence of DST must be established first. The binding and melting results are summarized in Table 1. As can be seen, ACTD binding to the self-complementary 14-mer d(ATATA-GGCC-TATAT) exhibits the weakest binding affinity ( $0.5 \times 10^6 \text{ M}^{-1}$ ), whereas that of d(ATATA-TGCA-TATAT) exhibits the strongest ( $8.4 \times 10^6 \text{ M}^{-1}$ ). Oligomers d(ATATA-CGCG-TATAT) and d(ATATA-AGCT-TATAT) exhibit moderate binding strengths of around  $2 \times 10^6 \text{ M}^{-1}$ . Consistent with the presence of a single binding site, all oligomers with ATATA-XGCY-TATAT motif show binding densities of roughly 1 drug molecule per duplex. These results are consistent with our earlier studies using these same -XGCY- sequences imbedded in a different sequence context, revealing that the order of binding for ACTD to be: -TGCA- > -CGCG- > -AGCT- >> -GGCC-. These results support the notion that ACTD indeed bind at the central -XGCY- sites of these oligomers. Studies have also been made with heteroduplexes d(GGCC-ATATA-CGCG)[d] and d(CGCG-ATATA-GGCC)[d]. Binding constants of roughly  $2 \times 10^6$  and binding densities of roughly 2 were obtained, consistent with ACTD binding at both sites of the duplex.

*DNA Binding of ACTD in the Presence of DST.* Similar ACTD binding titrations in the presence of 8  $\mu\text{M}$  DST were then carried out and the results are also included in Table 1 for comparison. Scatchard plots for oligomers of the form d(ATATA-XGCY-TATAT) show gross non-linearity so that the extraction of simple binding parameters from these plots was not feasible. Comparison of binding isotherms in the absence and in the presence of DST clearly suggests that the presence of DST greatly hinder the ACTD binding to these oligomers. This is particularly true for d(ATATA-AGCT-TATAT) and d(ATATA-TGCA-TATAT) (see Figure 1). In contrast to the progressive decrease in the apparent extinction coefficient of ACTD in the absence of DST, the apparent extinction coefficient of ACTD in the presence of DST remains largely unchanged until the [duplex] / [ACTD] ratio reaches around 0.5. The observed hindrance may be rationalized by the fact that in these two oligomers, the DST binding regions have been extended to 6-base duplexes, ATATAA and ATATAT, respectively, so that significant number of DST molecules will be bound to the inner 5-base region, possibly via 2:1 stoichiometries. Such binding will lead to interference with the ACTD binding at the AGCT and TGCA sites. This interpretation appears to be further supported by the binding results of heteroduplexes d(GGCC-ATATA-CGCG)[d] and d(CGCG-ATATA-GGCC)[d] where only about 2-fold reduction in binding affinities has been observed, conforming with the notion of less severe overlaps of the ACTD and DST binding sites in these two oligomers.

*DNA Binding of DST in the Absence of ACTD.* Results of equilibrium DNA binding studies of DST via CD titrations in the absence of ACTD are shown in Table 2. The estimated binding constants range from  $0.8 \times 10^6$  to  $1.5 \times 10^6 \text{ M}^{-1}$  and the binding densities are seen to be greater than 4. These results appear to be in conformity with the notion that DST binds at the two A•T track regions with 2:1 drug to DNA binding stoichiometries.

Similar binding strengths were also obtained for the heteroduplexes d(GGCC-ATATA-CGCG)[d] and d(CGCG-ATATA-GGCC)[d] but with considerably reduced binding densities, in agreement with the notion that now DST binds at the central A•T region.

*DNA Binding of DST in the Presence of ACTD.* DST binding titrations with DNA oligomers have also been carried out in the presence of 5  $\mu$ M ACTD. DST binding parameters for the d(ATATA-XGCY-TATAT) series do not appear to be greatly affected by the presence of ACTD, suggesting that a prior ACTD binding does not appear to greatly alter the subsequent DST binding at the A•T tracks. Interestingly, however, the prior binding of ACTD at the G•C regions results in the reduction of DST binding in d(GGCC-ATATA-CGCG)[d], whereas it increases the DST binding affinity in d(CGCG-ATATA-GGCC)[d]. More careful studies should be made before reaching any definitive conclusion.

*Results of Melting Experiments.* Binding of ligand to DNA usually results in enhancing its duplex stability. Thus, the relative melting temperature increases upon ligand binding may provide qualitative comparison of binding affinities. Melting temperatures of DNA in the absence and in the presence of 5  $\mu$ M ACTD are included in Table 1 for comparison. The relative melting temperature increases upon ACTD binding for the d(ATATA-XGCY-TATAT) series appear to be qualitatively consistent with the relative binding order established via previous binding titrations: -TGCA- > -CGCG-, -AGCT- > -GGCC-. Melting temperatures of oligomers in the presence of 8  $\mu$ M DST and 8  $\mu$ M DST + 5  $\mu$ M ACTD are included in Table 2 for comparison. Significant melting temperature increases were observed upon binding 8  $\mu$ M DST to oligomers of the form d(ATATA-XGCY-TATAT). In particular, melting temperature increases of 12 and 14 °C have been observed for the -TGCA- and -AGCT-containing oligomers. Interestingly, the additional presence of 5  $\mu$ M ACTD does not appear to further stabilize the DNA duplexes, suggesting some hindrance due to prior DST binding.

*Results of Kinetic Experiments.* ACTD association and SDS-induced drug dissociation kinetics in the absence and in the presence of 8  $\mu$ M DST have also been measured and the rate parameters extracted via single-exponential fits are shown in Table 3. Consistent with the weakest ACTD binding for d(ATATA-GGCC-TATAT), both ACTD association kinetics in the absence and in the presence of DST are too fast to be measurable by the non-stopped-flow technique. On the other hand, the rate of ACTD association with d(ATATA-CGCG-TATAT) is only slightly reduced by the presence of DST. Interestingly, however, nearly 3- and 8-fold reductions on the ACTD association rate are observed for d(ATATA-TGCA-TATAT) and d(ATATA-AGCT-TATAT), respectively. These results are consistent with the notion that A•T base pairs at the -TGCA- and -AGCT- sites are likely covered by DST so as to interfere with the ACTD binding at these sites. The kinetic results are consistent with the considerably larger melting temperature increases observed upon DST binding for these two oligomers, with the AGCT-containing oligomer exhibiting a larger melting temperature increase.

*CD Spectral Characteristics.* CD spectral alterations during a DST titration can reveal the mode of its DNA binding. Our earlier studies (Chen & Sha, 1998) have indicated that, in addition to a larger presence of the positive 330 nm CD band, a 2:1 DST binding mode is characterized by the appearance of a negative CD band near 290 nm and a positive CD intensity enhancement near 275 nm. CD difference spectra during DST titrations in the absence and in the presence of 5  $\mu$ M ACTD are compared in Figure 2 for d(ATATA-TGCA-TATAT) and d(ATATA-AGCT-TATAT). It is apparent that in the presence of

ACTD, stronger negative CD bands near 290 nm were observed. This is consistent with the notion that a prior ACTD binding at the central site has resulted in blocking off the innermost A•T base pairs for DST binding. In addition, the resulting stabilization of the 5-base-paired A•T regions led to a more favorable 2:1 mode of DST binding.

## CONCLUSION

Some interesting drug binding behaviors have been uncovered with the oligomeric sequences we have initially chosen. For example, a prior binding of DST to d(ATATA-AGCT-TATAT) or d(ATATA-TGCA-TATAT) greatly affects the subsequent binding of ACTD. This is the consequence of the fact that the A•T base pairs adjacent to the GpC site for ACTD has now become part of the DST binding site and are being occupied by the DST molecules. Thus, in order for ACTD to bind at the AGCT or TGCA site, these DST molecules must be displaced which requires a higher concentration of ACTD. As a consequence a rather unusual ACTD binding curve is observed during its titration in the presence of DST. A more suitable sequence for the synergistic studies may be oligomers such as d(GATATA-CCGCGG-TATATC) in which similar binding interference will be greatly reduced. The added G•C base pairs at the sequence terminals are for the purpose of reducing the end fraying effect so as to enhance the 2:1 mode of DST binding. On the other hand, the G•C base pairs adjacent to the CGCG site are to ensure minimal overlapping of the two kinds of drug bound. An appropriate sequence for synergistic binding studies of CHR and DST may be d(GATATA-CGGCCG-TATATC) since GGCC has been found to bind CHR tightly. A sequence such as d(GAAAAA-CCGCGG-TTTTC) or d(GAAAAA-CGGCCG-TTTTC) may be employed to study the synergistic effect of ACTD or CHR , respectively, with 1:1 mode of DST binding.

### Publications and manuscripts submitted during the past year:

Chen, F.-M. (1998) *Biochemistry* 37, 3955-3964. Binding of Actinomycin D to DNA Oligomers of CXG Trinucleotide Repeats.

Chen, F.-M. & Sha, F. (1998) *Biochemistry* 37, 11143-11151. Circular Dichroic and Kinetic Differentiation of DNA Binding Modes of Distamycin.

Sha, F., Mu, R., Henderson, D. and Chen, F.-M. *Biochemistry* (Submitted) Self-Aggregation of DNA Oligomers with XGG Trinucleotide Repeats, Kinetic and AFM Measurements.

Sha, F. and Chen, F.-M. *Biochemistry* (submitted) Actinomycin D Binds Strongly to d(CGACGACG) and d(CGTCGTG).

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Table 1. Actinomycin D binding parameters in the absence and in the presence of distamycin.

DNA Oligomer	K	n/dpx	K <sub>dst</sub>	n/dpx	t <sub>m</sub> <sup>o</sup>	t <sub>m</sub>
ATATA-GGCC-TATAT	0.5	0.9	*	*	49	52
ATATA-CGCG-TATAT	2.3	1.0	*	*	48	54
ATATA-AGCT-TATAT	1.9	1.1	*	*	43	47
ATATA-TGCA-TATAT	8.4	1.0	*	*	43	50
GGCC-ATATA-CGCG[d]	1.8	2.0	0.7	1.7	57	62
CGCG-ATATA-GGCC[d]	2.1	1.9	1.1	1.6	57	63

K and K<sub>dst</sub> are equilibrium binding constants (in  $\mu\text{M}^{-1}$ ) of ACTD at 20°C in the absence and in the presence of 8  $\mu\text{M}$  DST, respectively. T<sub>m</sub><sup>o</sup> and t<sub>m</sub> are melting temperatures (in °C) of DNA alone and in the presence of 5  $\mu\text{M}$  ACTD, respectively. (\*): unable to provide meaningful values due to the absence of linearity in Scatchard plots. Heteroduplex is designated by [d].

Table 2. Distamycin binding parameters in the absence and in the presence of actinomycin D.

DNA Oligomer	K	n/dpx	K <sub>actd</sub>	n/dpx	t <sub>m</sub> <sup>°</sup>	t <sub>m1</sub>	t <sub>m2</sub>
ATATA-GGCC-TATAT	0.8	4.4	0.7	6.8	49	57	53
ATATA-CGCG-TATAT	0.8	5.3	0.9	4.0	48	52	53
ATATA-AGCT-TATAT	1.5	4.7	1.0	5.0	43	57	57
ATATA-TGCA-TATAT	0.9	7.2	0.3	5.1	43	55	53
GGCC-ATATA-CGCG[d]	1.4	2.6	0.5	3.5	57	63	61
CGCG-ATATA-GGCC[d]	1.2	3.3	2.1	2.2	57	63	61

K and K<sub>actd</sub> are equilibrium binding constants (in  $\mu\text{M}^{-1}$ ) of DST at 20 °C in the absence and in the presence of 5  $\mu\text{M}$  ACTD, respectively. T<sub>m</sub><sup>°</sup>, t<sub>m1</sub>, and t<sub>m2</sub> are melting temperatures (in °C) of DNA alone, in the presence of 8  $\mu\text{M}$  DST, and in the presence 5  $\mu\text{M}$  ACTD + 8  $\mu\text{M}$  DST, respectively.

**Table 3.** Kinetic parameters at 20 °C for Actinomycin D in the Absence and in the presence of Distamycin.

DNA Oligomer	$k_a$ ( $\text{min}^{-1}$ )	$k_{a, \text{dst}}$ ( $\text{min}^{-1}$ )	$k_d$ ( $\text{min}^{-1}$ )	$k_{d, \text{dst}}$ ( $\text{min}^{-1}$ )
ATATA-GGCC-TATAT	*	*	0.39	
ATATA-CGCG-TATAT	3.0	2.2	0.23	0.23
ATATA-AGCT-TATAT	1.6	0.21	0.19	
ATATA-TGCA-TATAT	3.5	1.0	0.062	0.060
GGCC-ATATA-CGCG[d]	*	*	2.8	4.0
CGCG-ATATA-GGCC[d]	*	*	2.5	4.2

$k_d$  and  $k_{d, \text{dst}}$  are SDS-induced dissociation rate constants of ACTD in the absence and in the presence of 8  $\mu\text{M}$  DST, respectively. Kinetics which were too fast to be measurable by the non-stopped-flow technique are designated by (\*).

## FIGURE LEGENDS

Figure 1. Equilibrium binding isotherms for ACTD at 20°C, obtained by plotting  $\Delta A / [ACTD]$  vs.  $[DNA, duplex] / [ACTD]$  for d(ATATA-AGCT-TATAT) (upper panel) and d(ATATA-TGCA-TATAT) (lower panel) in the absence (solid symbols) and in the presence (open symbols) of 8  $\mu M$  DST.  $\Delta A$  is the absorbance difference between 427 and 480 nm.

Figure 2. Induced CD spectra of DST during titrations with d(ATATA-TGCA-TATAT) (left panels) and d(ATATA-AGCT-TATAT) (right panels) in the absence (upper panels) and in the presence (lower panels) of 5  $\mu M$  ACTD.

Figure 1

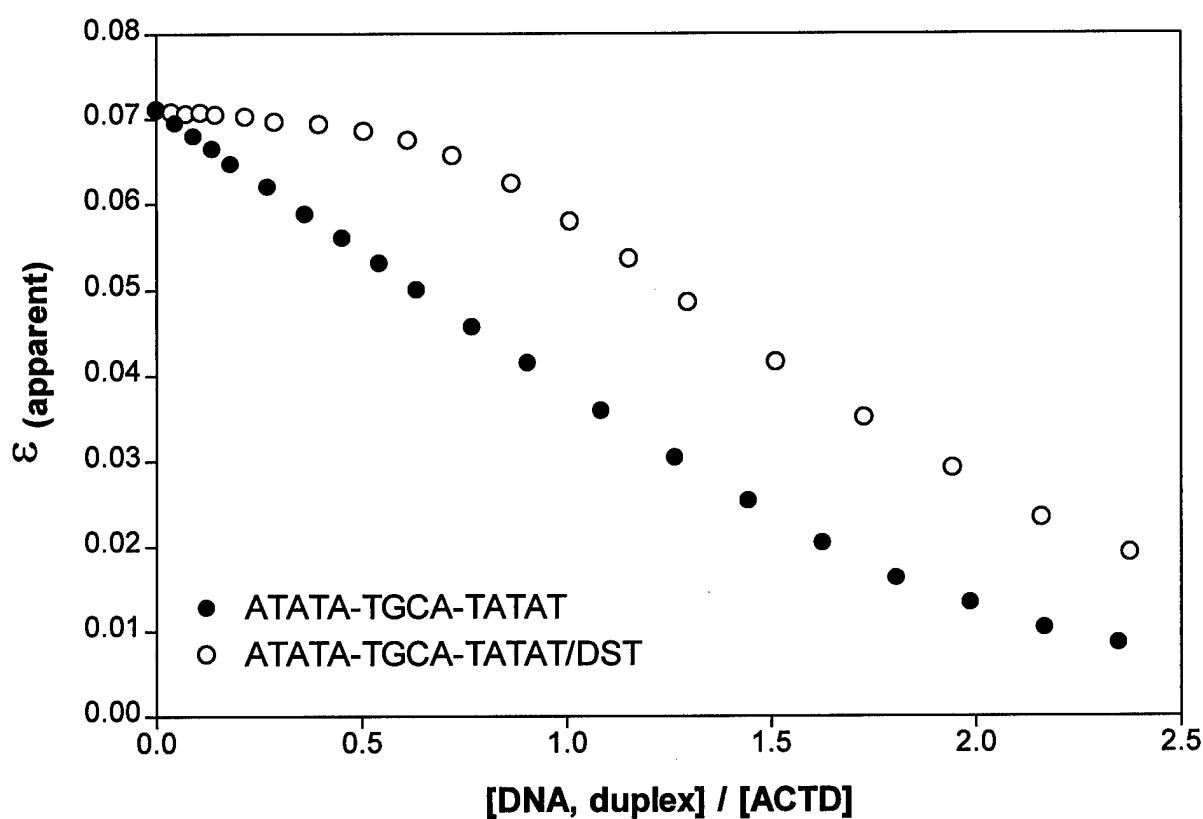
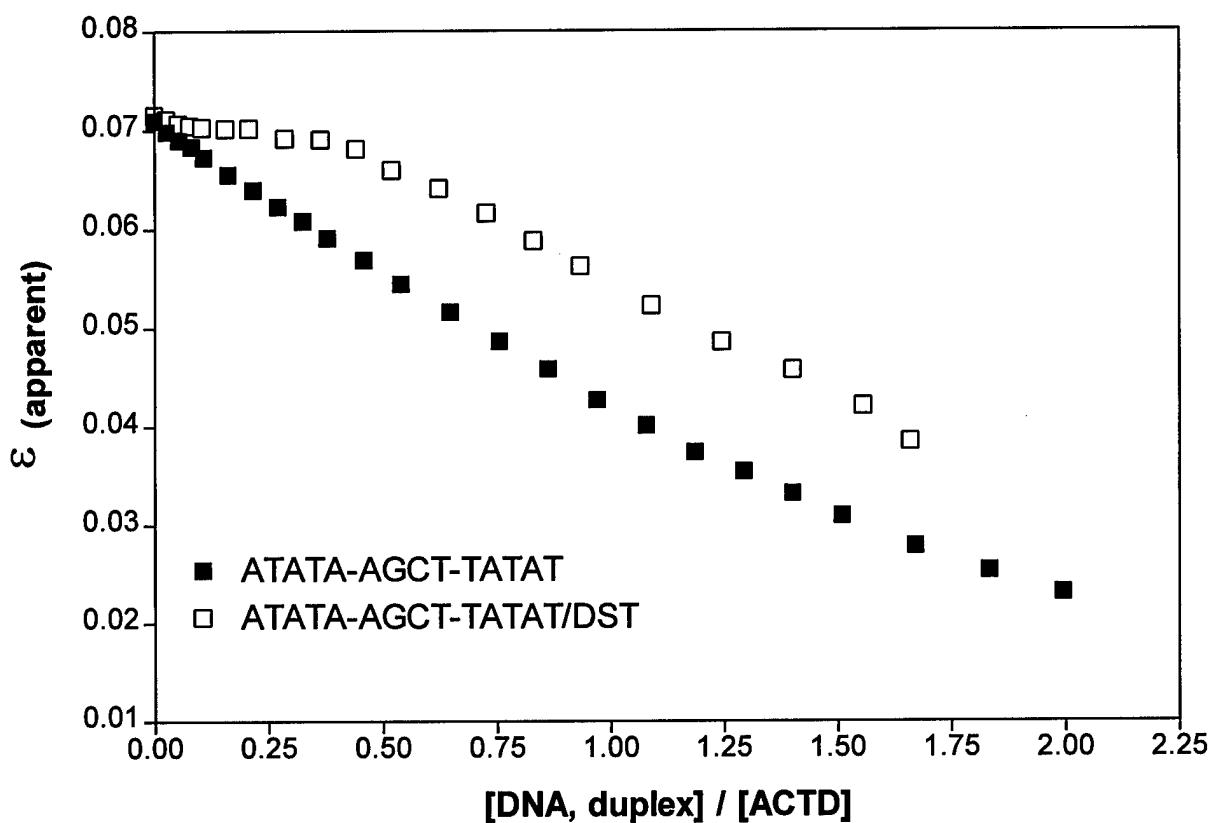


Figure 2

